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Flooding effects on soil microbial communities

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ABSTRACT

Flooding affects both above- and below-ground ecosystem processes. While the below-ground changes may be less obvious, they are as important as the above-ground changes. Soil microorganisms are sensitive to disturbance, and shifts in soil microbial community structure are expected when anaerobic conditions develop from flooding. The primary objective of these studies was to determine the effect of flooding on soil microbial communities. Simulated floods were established under greenhouse and field conditions. Flood treatments of flowing, intermittent (greenhouse only) or stagnant conditions were compared to a control with no flooding. In addition, residue treatments (incorporation of grass, legume or tree residue) were evaluated under greenhouse conditions. Soil samples from these experiments were examined using phospholipid fatty acid (PLFA) analysis and total N (TN), total organic carbon (TOC) and C:N ratio. Stagnant flood conditions in the greenhouse decreased microbial biomass and markers for aerobic bacteria, Gram-negative bacteria, Gram-positive bacteria, and mycorrhizal fungi. However, residue treatment, in general, did not affect microbial community structure. Effects of flood treatments in the field varied with depth and flood × depth interaction. The B:F ratio and microbial biomass decreased with stagnant flooding while other measures were not affected by flooding. Microbial biomass and microbial markers decreased with depth. We found some changes in the soil microbial community due to flooding; however, the 5-week time period of our study may not have been long enough to develop measureable changes. Further changes in the microbial community may occur as flood waters remain in a given area.

1. Introduction

Active floodplains have a flooding recurrence interval of once per year on average (Bridge, 2003). However, heavy or prolonged rain events may result in soil saturation, particularly in concave or low-lying landscape features. When combined with microbial activity, soil inundation or saturation depletes soil oxygen. This loss of soil oxygen is the greatest challenge to established plants. Plants tolerant of flooding have specific physiological and anatomical adaptations that allow them to cope with diminished soil oxygen; while flood-intolerant plants lack such adaptations. With the loss of soil oxygen, microbes capable of anaerobic respiration will shift to alternative electron acceptors for their metabolic needs. This results in reduced forms of oxygen, nitrogen, manganese, iron or sulfur in the soil. Reduction reactions may result in changes in phase or solubility. For example, reduced Fe and Mn are more soluble; these reduced ions move through the soil resulting in areas with either a depletion or surfeit of Fe and Mn (Vepraskas and Faulkner, 2001). Soluble forms of Fe and Mn are more available to plants, and toxic concentrations are possible. On the other hand, reduction of NO_3^- results in a phase change, transforming available N into various gaseous forms (i.e. N_2, N_2O, and NO_2) which can escape the soil environment resulting in potential N depletion (Vepraskas and Faulkner, 2001). Several studies revealed that longer inundations result in anoxic/anaerobic conditions, thereby decreasing decomposition and altering nutrient cycling (Baker et al., 2001; Baldwin and Mitchell, 2000; Neatrour et al., 2004; Schuur and Matson, 2001). For example, the decomposition of lignin proceeds slowly under anoxic conditions and in some cases (e.g., rice systems) this results in the production of phenolic acids (Olk et al., 1996; Tsutsuki and Ponnamperuma, 1987) some of which are thought to be alleopathic.

A disturbance such as flooding affects both above- and below-ground ecosystem processes. Although often ignored, changes in below-ground environments following flooding are no less important than those that occur above-ground. Cropping, tillage and other land management practices can have significant effects on soil microbial community characteristics (Suzuki et al., 2005;

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Fraterrigo et al., 2006). Conventional and no-till farming systems may support different soil microbial communities (Petersen et al., 2002) and the application of chemical fertilizers may alter the bacterial:fungal ratio of a given community (Suzuki et al., 2005). Shifts in soil microbial community structure are expected when anaerobic conditions develop from flooding (Elhottova et al., 2002; Mentzer et al., 2006). These changes may subsequently affect above-ground components of the ecosystem due to the critical roles that bacteria and fungi play in decomposition and nutrient cycling (Suzuki et al., 2005).

Understanding the effects of disturbance on the soil microbial community is met with the challenge of quantifying that community. Phospholipid fatty acid (PLFA) analysis uses membrane phospholipids for microbial community characterization and represents one method that overcomes the problem of selective culturing (Petersen and Klug, 1994) and has been used to characterize soil microbial communities in flooded soils (Hackl et al., 2005; Bossio et al., 2006). The primary objective of this experiment was to determine the effect of various flood treatments on soil microbial community structure. Similar to plants, microbial tolerance to flooding and anaerobic conditions vary; therefore, microbial community structure is expected to change with flooding. A secondary objective to determine the effect of various residue treatments on soil microbial community structure was included in the greenhouse study. Inclusion of residue is expected to increase microbial biomass over the control; however, no changes in microbial structure with residue type are expected.

2. Methods

2.1. Greenhouse study

Soil for the greenhouse experiment was collected from the Flood Tolerance Laboratory (FTL) at the University of Missouri Horticulture and Agroforestry Research Center (HARC) in New Franklin, MO (39°0′0″N, 92°46′0″W). Soils in this floodplain are generally classified as Nodaway silt loam, occasionally flooded (fine-silty, mixed, superactive, nonacid, mesic Mollic Udifluvents). Soil from the upper 20 cm was collected; air dried; sieved (2 mm); and stored cold (10 °C) in plastic-lined bins until use. The greenhouse experiment investigated the effects of both flood and residue treatments on the soil microbial community in a randomized complete block with four replications. The four flood treatments that replicated natural flood regimes were: (1) saturated and stagnant (stagnant treatment); (2) saturated and flowing (flowing treatment); (3) periodic saturation and then drained (intermittent treatment); and (4) control treatment at 30% soil water content (SWC). Four residue treatments were used: (1) tree (swamp white oak, *Quercus bicolor*); (2) legume (false wild indigo, *Amorpha fruticosa*); (3) grass (manna grass, *Glyceria striata*); and (4) control with no residue incorporated. The species selected were native to Missouri and are typically found in riparian or floodplain habitats. All plant samples were dried (40 °C, 2–3 days) and ground to pass a 1-mm sieve.

The treatments were established in clear plastic trays (22 cm × 35 cm) with opaque lids. Trays were lined with white 13-gallon trash bags to reduce the amount of light entering the tray and to reduce algal and moss growth. To each tray, 9.2 kg of soil was added to make layer of soil 10 cm deep. Plant residues (either grass, legume or tree species) were incorporated into the soil by hand at 8 Mg residue ha^{-1}. Flood treatments followed the incorporation of plant residue. The control treatment consisted of 2.5 L of de-ionized water added to the soil/residue mixtures. Water was added back as needed to maintain tray weight and 30% SWC. The flooding treatments consisted of 5 cm of water above the soil surface (Cirtain et al., 2004). The water in the stagnant treatment was undisturbed. The flowing water conditions were created by pumping the water through the trays at 2.73 L m^{-1}. The intermittent treatment consisted two cycles of a 2-week flooding period and 2-week dry-down period (surface water removed by bailing and draining). Four replications of each flood × residue combination were established for a total of 64 trays.

The treatments lasted 56 days (December 19, 2005 to February 14, 2006) with 9 h days and 15 h nights and air temperatures ranging from 17 to 20 °C and soil temperatures of 16 to 18 °C. After 56 days, two 3.8 cm diameter cores were taken from the center of each tray. These cores were combined and samples were placed in plastic bags, frozen and later freeze dried.

2.2. Field flood experiment

The field portion of the study was conducted at the Flood Tolerance Laboratory (FTL) at the University of Missouri Horticulture and Agroforestry Research Center in New Franklin, MO (39°0′0″N, 92°46′0″W). The FTL is an outdoor research facility constructed on a wide terrace floodplain adjacent to Sulphur Creek. The FTL consists of 12 6 m × 180 m parallel channels; each channel is manipulated independently to allow for various flood treatments (i.e. changing depth, flow rate and duration of flooding). Three flood treatments at the FTL were evaluated: (1) no flood (control); (2) 5 weeks of flowing water 15 cm (flowing); and (3) 5 weeks of stagnant water maintained at 15 cm (stagnant). The experimental design was a randomized complete block with three blocks arranged in a north-south direction; each block contained each of the four treatments. Experimental channels were flooded on May 23, 2005 and drained on June 27, 2005. For the FTL field experiment, 10 soil cores (2.5 cm diameter) were collected from the eastern third of each channel (an area of approximately 4 m × 60 m) at two depths: 0–10 cm and 10–20 cm and on two collection dates: May (pre-flood) and July (post-flood). Cores were pooled to create a single sample for each channel for each date × depth combination. Mixing of the samples in this matter corrects for heterogeneous soil conditions across the sampling area. Samples were placed in plastic bags on ice and frozen once back in the lab.

2.3. Soil analyses

For analyses, soil samples were freeze-dried in a production-grade freeze drier at −20 °C and 4.0 Torr and ground to pass through a 2 mm sieve. Samples were analysed by dry combustion for total N (TN) and total organic C (TOC) using a LECO TruSpec CN analyzer (St. Joseph, MI); C:N ratios were calculated from these measurements.

2.4. Microbial community structure

Whole soil phospholipid fatty acid procedures generally follow Bligh and Dyer (1959) as described by Petersen and Klug (1994). All reagents were HLPC Grade and purchased from Sigma (St. Louis, MO) except where noted. Soil samples (2 g) were placed in Teflon-lined screw cap culture tubes (16 mm × 100 mm) and fatty acid methyl esters analysis was conducted based on saponification of soil at 100 °C, acid methylation at 80 °C, an alkaline wash, and an extraction of methyl esters of long-chain fatty acids and similar lipid compounds into hexane. Nonadecanoic acid methyl ester was included after the methylation step for to enable quantification of identified lipids on a molar basis. Samples for phospholipid analysis were separated by solid phase extraction using 100 mg silica columns (Varian, Palo Alto, CA). Columns were conditioned with 3 mL hexane, 1.5 mL hexane/chloroform (1:1) and 100 μL chloroform and a slight vacuum (1–2 in Hg) was applied to the

columns after the addition of each solvent. The columns were rinsed through the sequential addition of 1.5 mL chloroform/2-propanol (1:1) and 1.5 mL 2% acetic acid in diethyl ether with vacuum. Finally, phospholipids were eluted from the columns with 2 mL methanol, and evaporated under nitrogen in preparation of extraction of the PLFAs. The combined organic phase was evaporated to dryness under nitrogen and then redissolved in 75 μL hexane:methyl tertiary butyl ether (1:1).

Fatty acid methyl esters were analysed on a gas chromatograph (Agilent Technologies GC 6890, Palo Alto, CA) with a fused silica column and equipped with a flameionizer detector and integrator. ChemStation (Agilent Technologies GC 6890, Palo Alto, CA) operated the sampling, analysis, and integration of the samples. Peak identification and integration of areas were performed under the Eukary method parameters by software supplied by Microbial Identification Systems, Inc. (Newark, DE). Peak chromatographic responses were translated into mol responses using the internal standard and responses were recalculated as needed.

Peaks that correspond to carbon chain lengths of 12–20 carbons are generally associated with microorganisms. Bacterial:fungal ratios were calculated for each sample. Peaks used as markers for bacteria were 12:0 3OH, *i*14:0, 15:0, *a*15:0, *i*15:0, *i*15:0 g, *cyc*15:1, *i*16:0, 16:1ω7, *cis*16:1ω7, *trans*16:1ω7, *a*17:0, *cy*17:0, *i*17:0, 17:1ω6, *i*17:1ω7, 18:1ω7, *cis*18:1ω7, *cis*18:1ω9, *cyc*19:0, *cyc*19:0 C11-12, *cy*19:0, *cis*19:1ω9 (Vestal and White, 1989). Fungal markers were 16:1ω5, *cis*16:1ω5, 18:1ω9, 18:2ω6, *cis*18:2ω6, 18:2ω9, 18:3ω3, 18:3ω6, *cis*18:3ω6 (Federle, 1986; Wander et al., 1995; Zelles et al., 1995; Frostegard et al., 1993; Sundh et al., 1997). For Gram-positive bacteria, markers were *i*14:0, *i*15:0, *a*15:0, *i*15:0 g, *i*16:0, *i*17:0, *cis*18:1ω9 (O'Leary and Wilkinson, 1988; Wander et al., 1995; Zelles et al., 1995; Sundh et al., 1997). The markers for Gram-negative bacteria were 15:1ω6c, *cis*16:1ω7t, *cy*17:0, *cis*18:1ω7; *cy*19:0, *cyc*19:0, *cis*19:1ω9 (Ratledge and Wilkinson, 1988; Wander et al., 1995; Zelles et al., 1995; Sundh et al., 1997). Mycorrhizal markers were 16:1ω5, *cis*16:1ω5, 18:2ω6, *cis*18:2ω6, 18:2ω9 (Balser et al., 2005; Belen Hinojosa et al., 2005).

Stress indicators were calculated based on the ratios of the cyclopropyl fatty acids to monoenoic precursors and the total saturated to total monounsaturated fatty acids (Kieft et al., 1997; Bossio and Scow, 1998; Fierer et al., 2003). Specific peaks used to calculate the cyclopropyl fatty acids to monoenoic precursor ratios were *cy*17:0 to *cis*16:1ω7 and *cy*19:0 to *cis*18:1ω7. The ratio of total saturated to total monounsaturated fatty acids used the ratio of the sum of 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0 to sum of *cis*16:1ω11, *cis*16:1ω9, *cis*16:1ω7, *cis*16:1ω5, *cis*17:1ω9, *cis*17:1ω8, *cis*17:1ω7, and *cis*17:1ω5. Monounsaturated fatty acids from 14:0 to 19:0 were also evaluated (Bossio and Scow, 1998). Biomass was calculated using the relationship determined by Bailey et al. (2002).

2.5. Data analysis

Percentages of fatty acids covered a wide range of values and were log transformed for principal component analysis (PCA) in SAS (2002). Principal component analysis was used as exploratory data analysis to reduce the dimensionality in the data and to examine the associations in the microbial populations (Tabachnick and Fidell, 2001). Data were presented as a 2D plot for better understanding of the relationship. We used Kaiser's rule (Joliffe, 1986), that only variables with an eigenvalue greater than one are to be used for further analysis when the correlation matrix is used in PCA. In our initial analyses, we computed the correlation between principal components and fatty acids for PC1, PC2, PC3, and PC4. In PCA, the eigenvectors determine the directions of maximum variability and the eigenvalues specify the variances.

For soil samples from the simulated flood experiment, two-way ANOVA (Proc GLM) was used to test the effects of flood and residue treatments on: (i) PLFA PCA axes 1 and 2, (ii) bacterial:fungal (B:F) ratio and total microbial biomass, (iii) responses due to various microbial markers, and (iv) stress indicators and monounsaturated fatty acids. Significant differences led to pairwise comparisons (Fisher's Least Significant Difference (LSD), $\alpha = 0.05$). For soil samples from the FTL, one-way ANOVA (Proc GLM) was used to analyse the effects of sampling date, sample depth and flood treatment on: (i) PLFA PCA axes 1 and 2, (ii) B:F ratio and total microbial biomass, (iii) responses due to various microbial markers, and (iv) stress indicators and monounsaturated fatty acids. For the flood treatment analyses, each sample date (pre-and post-flood) and depth (upper (0–10 cm) and lower (10–20 cm)) combination was tested separately. Pearson correlation coefficients were calculated to determine the relationships among soil chemical characteristics (TOC, TN, and C:N ratio) and soil microbial community characteristics. All analyses were conducted using SAS 9.1 SAS (2002).

3. Results

3.1. Greenhouse experiment

Principal component analysis of PLFAs revealed flood treatment effects, but no residue effects on the soil microbial community (Fig. 1). PC1 accounted for 53% of the variance and PC2 was responsible for explaining 18% of the data variance. Soil samples separated into groups that aligned with flood treatments. Aerobic flood treatments (i.e. control and intermittent flood treatments) and anaerobic flood treatments (i.e. flowing and stagnant flood treatments) separated along the PC1 axis. Separation along the PC1 axis was due to 15:0, *cy*17:0, 16:1ω7c, 16:0 and 18:3ω6c, while separation along the PC2 axis was due to *a*17:1; *cy*19:0, and 18:1ω9t. In general, fungal components were responsible for separation on both PC 1 and 2 and bacterial components were responsible for PC1 separation.

ANOVA of PC1 revealed a significant flood effect and flood × residue interaction ($p < 0.0001$ and $p < 0.01$ respectively); a significant effect due to residue treatment was not observed for this axis ($p = 0.62$). Fisher's LSD analysis of PC1 revealed that the intermittent and control treatments were significantly different from the flowing and stagnant treatments. ANOVA on PC2 revealed

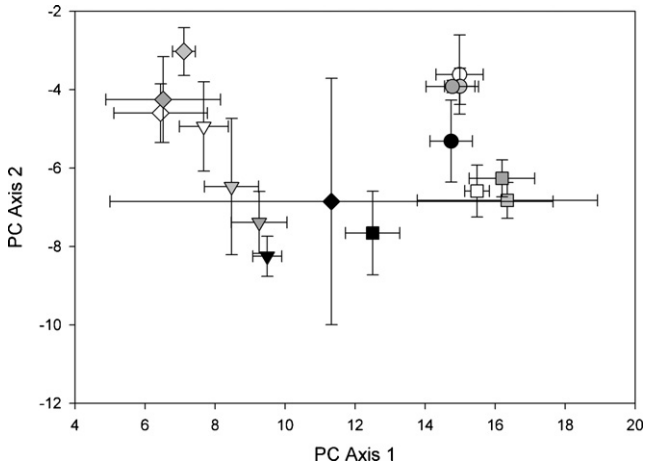

Fig. 1. Principal component analysis of phospholipid fatty acids from a Nodaway silt loam under three flood (stagnant = diamonds, flowing = triangles or intermittent = squares) and three residue (tree = dark grey, legume = light grey or grass = white) treatments and controls (control flood = circles; control residue = black symbols) over a 56-day period in a greenhouse experiment. PCA axis 1 explains 53% and axis 2 explains 18%.

Table 1
Mean values (and standard deviations) for soil microbial community characteristics and soil chemical analysis from phospholipid fatty acid analysis of a Nodaway silt loam subjected to three flood (stagnant, flowing or intermittent) and three residue (tree, legume, grass) treatments (data not shown) and controls over a 56-day period in a greenhouse experiment. Flood treatment means with the same letter are not significantly different ($\alpha = 0.05$).

Flood treatment	Microbial biomass(mg C g^{-1} soil)	%									
		Aerobic bacteria	Anaerobic bacteria	Gram-negative bacteria	Gram-positive bacteria	Mycorrhizal fungi	Stress indicators	Mono-unsaturated fatty acids	TN	TOC	C:N ratio
Control	154.70 b (23.12)	6.58 b (0.41)	5.86 (0.98)	5.65 a (0.35)	19.36 b (3.28)	5.61 a (1.03)	1.45 b (0.23)	0.18 a (0.01)	0.14 (0.02)	1.34 (0.10)	9.75 (1.43)
Intermittent	189.50 a (73.81)	9.08 a (1.55)	6.28 (1.02)	4.04 b (0.65)	8.71 c (1.16)	4.01 c (0.43)	1.93 ab (1.44)	0.19 a (0.01)	0.15 (0.01)	1.37 (0.08)	9.21 (0.67)
Flowing	183.34 ab (43.83)	5.53 b (0.85)	6.57 (0.74)	3.48 b (0.75)	22.60 a (3.91)	3.07 b (1.11)	2.19 a (0.34)	0.19 a (0.01)	0.14 (0.02)	1.30 (0.32)	9.16 (2.47)
Stagnant	83.28 c (27.10)	4.13 c (2.51)	6.55 (3.39)	1.73 c (0.87)	8.50 c (0.51)	2.67 c (1.45)	2.41 a (1.21)	0.16 b (0.04)	0.14 (0.01)	1.32 (0.10)	9.80 (0.125)

Note: TN = total N; TOC = total organic C; C:N = carbon to nitrogen ratio.

a significant flood effect ($p < 0.0001$ and $p < 0.0001$ respectively), but no interaction ($p = 0.10$). Control and stagnant flood treatments were significantly different from flowing and intermittent flood treatments on PC2; likewise, the control residue treatment was different from the other residue treatments.

Flood treatment had the greatest impact on the soil microbial community structure. Microbial biomass, markers for aerobic bacteria, Gram-negative bacteria, Gram-positive bacteria, and mycorrhizal fungi, the stress indicators and the sum of mono-unsaturated peaks all varied with flood treatment (Table 1). A significant flood × residue interaction was observed for the B:F ratio (Table 2). Biomass was lowest under stagnant flood conditions (Table 1). The intermittent flood treatment resulted in the greatest response for the aerobic bacteria, while the stagnant flood treatment showed the least (Table 1). The flowing and control flood treatments were not different in terms of mol percent response for aerobic bacteria.

Gram-negative bacteria and mycorrhizal fungi markers responded similarly to flood treatment (Table 1), with the control treatment having the greatest response and the stagnant treatment resulted in the lowest response. The intermittent and flowing flood treatments did not differ; these flood treatments resulted in an intermediate response for these microbial markers (Table 1). Gram-negative bacteria and Gram-positive bacteria as well as mycorrhizal fungi were negatively affected by anaerobic conditions brought about by stagnant flood waters. Gram-positive bacteria were most prevalent under flowing flood and control conditions and least prevalent under intermittent and stagnant flood conditions (Table 1). Gram-negative bacteria differed with residue treatment (data not shown). The greatest response for Gram-negative bacteria was observed with grass residue; the other residue treatments showed lower response and were not significantly different from each other.

A significant flooding × residue interaction occurred with the B:F ratio (Table 2). The highest B:F ratios were observed for the control flood × residue combinations, as well as for the flowing flood/control residue treatment. Other flowing flood × residue combinations, as well as all intermittent flood × residue combinations and all stagnant flood × residue combinations resulted in significantly lower B:F ratios (Table 2).

Table 2
Average B:F ratio determined from phospholipid fatty acid analysis of a Nodaway silt loam subjected to three flood (stagnant, flowing or intermittent) and three residue (tree, legume, and grass) treatments and controls over a 56-day period in a greenhouse experiment. Flood treatment means with the same letter are not significantly different ($\alpha = 0.05$).

Experimental treatment		B:F ratio	S.D.
Flood	Residue	Average	
Control	Control	1.94 bc	0.15
	Grass	1.93 bc	0.14
	Legume	1.69 c	0.08
	Tree	2.57 a	0.82
Intermittent	Control	0.39 de	0.04
	Grass	0.52 de	0.07
	Legume	0.57 de	0.09
	Tree	0.60 d	0.07
Flowing	Control	2.19 b	1.23
	Grass	0.32 de	0.03
	Legume	0.34 de	0.04
	Tree	0.34 de	0.01
Stagnant	Control	0.56 de	0.22
	Grass	0.45 de	0.06
	Legume	0.46 de	0.10
	Tree	0.31 e	0.21

Table 3
Mean values (and standard deviations) for soil microbial community characteristics and soil chemical analysis from phospholipid fatty acid analysis after various *in situ* flood (control, 5 weeks of flowing water, or 5 weeks of stagnant water) treatments at the Flood Tolerance Laboratory, New Franklin, MO. Values in the same column with the same letter are not significantly different ($\alpha = 0.10$).

Depth (cm)	Flood treatment	B:F	Biomass (mg C g^{-1} soil)	%							
				Aerobic bacteria	Gram-negative bacteria	Gram-positive bacteria	Mycorrhizal fungi	Stress indicators	Mono-unsaturated fatty acids	TN	TOC	C:N
0–10	Control	2.04a (0.6)	139c (36.7)	61.7b (15.7)	92.1b (15.8)	189.9b (40.3)	81.0ab (83.1)	2.04b (0.2)	0.17 (0.01)	0.13 (0.02)	1.22 (0.2)	9.51a (0.5)
0–10	Flowing	1.92b (0.3)	165b (27.8)	80.6a (18.0)	91.8b (21.5)	238.3a (39.8)	89.7a (5.4)	2.02b (0.2)	0.2 (0.01)	0.13 (0.01)	1.25 (0.1)	9.33ab (0.1)
0–10	Stagnant	1.84b (0.6)	159b (71.0)	80.0a (47.6)	85.5b (35.3)	220.8ab (125.5)	99.6a (50.3)	1.94b (0.4)	0.17 (0.02)	0.13 (0.03)	1.23 (0.2)	9.12b (0.3)
10–20	Control	2.44a (0.6)	123c (27.5)	49.7c (6.7)	87.3b (15.1)	165.8c (25.3)	59.5c (14.9)	2.09b (0.5)	0.17 (0.03)	0.12 (0.01)	1.08 (0.1)	9.11a (0.4)
10–20	Flowing	2.29a (0.2)	183a (66.3)	66.9b (15.4)	122.2a (14.9)	231.0a (82.7)	82.5ab (19.5)	2.46a (0.4)	0.19 (0.02)	0.12 (0.01)	1.06 (0.1)	8.75b (0.2)
10–20	Stagnant	1.73b (0.7)	92d (23.6)	33.2c (8.7)	63.1c (14.8)	108.8d (26.5)	64.5bc (23.4)	2.51a (0.3)	0.17 (0.02)	0.11 (0.01)	1.03 (0.2)	9.18a (0.6)

Note: B:F = bacterial to fungal ratio; TN = total N; TOC = total organic C; C:N = carbon to nitrogen ratio; flowing = 5-week-flowing flood; stagnant = 5-week-stagnant flood.

Variation observed in stress indicators and in monounsaturated fatty acids were due primarily to flood treatment. There were no differences due to residue treatment or a flood - × residue interaction. Stress indicators were significantly higher under stagnant and flowing flood conditions than under control conditions (Table 1). The intermittent treatment had intermediate levels of stress indicators and was not different from the other flood treatments or from the control. The monounsaturated fatty acids were lowest under stagnant flood conditions; however, other treatments were not different for this microbial component (Table 1). Flood treatments did not affect TN, TOC or the C:N ratio (Table 1).

3.2. Field experiment

PCA analysis of PLFA from soils of the FTL field study revealed that the greatest variation was due to sampling depth with only a few differences seen among the control soils and flowing or stagnant conditions (data not shown). An examination of the post-flood samples from the 5-week flood treatments revealed a decrease in microbial parameters with sampling depth and flood treatment (Table 3). At the 0–10 cm depth, the flood treatments reduced bacterial to fungal ratios by 4% for the flowing flood treatment and by almost 10% for the stagnant flood treatment compared to the control soil. Biomass values were lower for the control soils at 139 mg C g^{-1} soil; the flooding treatments were 165 mg C g^{-1} soil for flowing and 159 mg C g^{-1} soil for stagnant. Surprisingly, aerobic bacterial markers were higher in the flood-treated soils than the control soils. The flowing flood treatments had more Gram-positive bacterial markers than the control soils. All other markers and TN, TOC and C:N were not significantly different from the control at the 0–10 cm depth.

Microbial components at the lower depth (10–20 cm) in the FTL field study were variable in their response (Table 3). Bacterial to fungal ratios were highest in control and flowing treatments compared to the stagnant treatment, which was 29% less than the control. Biomass values were highest for the flowing flood treatment and lowest for control and stagnant. Aerobic bacterial markers were highest in the flowing treatment followed by control soils with stagnant soils having the least aerobic bacterial markers. Gram-negative bacterial and Gram-positive bacterial markers were greatest in the control and flowing flood treatment and least with stagnant flood treatment. Mycorrhizal markers were highest in flowing and lowest in control. Stress indicators were low in control and highest in flowing and stagnant treatments. C:N ratios at the lower depth were highest for control and stagnant and lowest for flowing. Monosaturated markers, TN and TOC were not different among the treatments.

Table 4
Pearson correlations of TN, TOC and C:N ratio with soil microbial community characteristics of B:F ratio, biomass, and markers for aerobic bacteria, Gram-negative bacteria, Gram-positive bacteria, mycorrhizal fungi, and stress indicators from phospholipid fatty acid analysis after various *in situ* flood treatments (control, 5 weeks of flowing water, or 5 weeks of stagnant water) at the Flood Tolerance Laboratory (FTL), New Franklin, MO. Significant values are indicated as follows: $\alpha < 0.05 = {}^{*}$, $\alpha < 0.01 = {}^{**}$ and $\alpha < 0.001 = {}^{***}$.

Microbial component	TN	TOC	C:N ratio
B:F ratio	−0.04	0.03	0.21
Microbial biomass	0.40^{**}	0.35^{**}	−0.06
Aerobic bacteria	0.53^{***}	0.51^{***}	0.06
Gram-negative bacteria	0.41^{**}	0.39^{**}	0.03
Gram-positive bacteria	0.41^{**}	0.40^{**}	0.02
Stress indicators	-0.29^{*}	-0.39^{**}	-0.37^{**}

3.3. Correlations: soil microbial structure with TOC and TN

The correlation between soil microbial structure and TOC and TN differed for the experiments. For the greenhouse experiment, soil community structure was largely unrelated to soil C and N (data not shown). Several correlations were observed for the FTL field samples (Table 4). Biomass, along with the markers for aerobic bacteria, Gram-negative bacteria and Gram-positive bacteria were positively correlated with both TOC and TN. On the other hand, stress indicators were negatively correlated with TOC, TN and the C:N ratio. No correlations with TOC, TN or C:N ratio were observed for the B:F ratio, monounsaturated fatty acids, or the mycorrhizal fungal markers. The microbial markers were not correlated with C:N ratio.

4. Discussion

The microbial communities of the soils from the greenhouse and field studies varied in marker composition and in response to flood disturbances. Samples from the greenhouse experiment showed a change in community structure with flooding; however, samples from the field study showed a greater influence from depth than flood treatments. Similarly, Ibekwe and Kennedy (1998) described differences between greenhouse and field soil samples with higher % PLFA composition and higher microbial biomass in field soils rather than greenhouse soils.

The greenhouse experiment revealed changes in microbial community structure with flooding and less so with residue addition. Residue treatments may have affected TN and TOC levels early in the flood treatments and thus may have affected soil microbial community structure initially. However, soil samples for PFLA analysis were taken at the end of the 56-day flood treatments and at this time, soil TN and TOC were no longer different despite different residue treatments. Other studies have shown similar responses (Drenovsky et al., 2004; Mentzer et al., 2006). Mentzer et al. (2006) found that prolonged flooding had a greater effect than nutrient loading in that flooding altered both the composition as well as functional components of the microbial community. Specifically, flooding reduced the mycorrhizal fungal markers, while increasing Gram-negative bacterial, anaerobic bacterial and Gram-positive bacterial markers. Drenovsky et al. (2004) also observed a decrease in fungal biomarkers, but not bacterial biomarkers with increased soil water content. Furthermore, Bossio and Scow (1998) observed a decrease in fungal and aerobic indicators and an increase in Gram-positive bacterial indicators with flooding; however, they also observed changes in microbial indicators with straw incorporation. The decreased presence of fungi under flooded conditions observed in the current study and other studies (Bossio and Scow, 1998; Drenovsky et al., 2004; Mentzer et al., 2006) is consistent with the hypothesis that fungi are less prevalent in inundated soils.

Analysis of the FTL field soil samples revealed the importance of sampling depth. General decreases in biomass and reduced response of microbial markers were observed with increased sampling depth. Bacteria are typically most numerous in surface layers that are rich in organic material or in the rhizosphere where plant roots release sugars, amino acids and other organic compounds. In addition, mycorrhizae are associated with plant roots that will likewise be concentrated in the upper soil layers. A soil profile analysis conducted by Fierer et al. (2003) demonstrated the changes in soil microbial community structure with depth. An overall decline in microbial diversity was detected by Fierer et al. (2003) along with declines in individual PLFA markers. Not all groups responded consistently. Gram-negative bacterial and fungi markers declined with depth; while Gram-positive bacterial markers increased with depth (Fierer et al., 2003). Others have noted a decline in microbial biomass and changes in PLFA markers with depth (Fritze et al., 2000; Peacock et al., 2001). In both of these cases, microbial biomass was greatest at the surface; for example, Peacock et al. (2001) observed that microbial biomass was twice as great in the surface layers (0–5 cm) than at 5–10 cm or 10–15 cm. The authors relate the declines in microbial community diversity and biomass with depth to changes in soil nutrient status with depth (Fritze et al., 2000; Peacock et al., 2001; Fierer et al., 2003). In the current study, an increase in depth of 10 cm (0–10 cm vs. 10–20 cm sampling depth) resulted in a 9% decrease in microbial biomass as well as a 23% decrease in aerobic bacterial markers, a 5% decrease in Gram-negative bacterial markers, a 15% decrease in Gram-positive bacterial markers and a 12% decrease in mychorrizal fungal markers. The same increase in depth resulted in a 12–13% decrease in TOC and a 9–10% decrease in TN. Carbon inputs are thought to decrease not just in availability, but also in quality with depth (Fierer et al., 2003). Carbon enters the soil profile primarily through leaf litter or plant residues on the surface or via root exudates in the upper soil horizons. As litter and residues are broken down first by macro- and micro-fauna and later by bacteria and fungi, carbon is transferred through the soil profile. The more labile carbon products are removed first, while the more recalcitrant carbon products are passed further down, through the soil profile. Some microbial groups prefer the more readily available forms of carbon such as sugars and amino acids while other groups prefer the more recalcitrant compounds such as lignin and cellulose.

The stress indicators include the ratio of the relative abundance of cyclopropyl fatty acids to their monoenoic precursors; the abundance of cyclopropyl fatty acids is used as an indicator of anaerobic conditions (Bossio and Scow, 1998). While the current study revealed increases in stress indicators with flooding in the greenhouse, other studies have failed to show such a relationship. Bossio and Scow (1998), for example, found no changes in cyclopropyl fatty acids due to flooding and branched fatty acids were more prevalent in wetland soils compared to agricultural soils (Bossio et al., 2006). Meanwhile, increases in cyclopropyl fatty acids observed by Fierer et al. (2003) were associated with increased soil depth (as was observed in the FTL field study). Fierer et al. (2003) speculated that deeper soil horizons have more severe resource limitations than surface soil horizons resulting in shifts in microbial membrane fatty acids.

While the response of the stress indicators was similar to patterns observed for the other microbial markers (i.e. affected by flood treatment in the greenhouse and by depth in the FTL field study), the response of monounsaturated fatty acids is less clear. Monounsaturated fatty acids are strongly related to higher substrate availability (Bossio and Scow, 1998); therefore, this measure should decrease under more stressful conditions. In the greenhouse flood experiment, the stagnant flood treatment resulted in significantly lower monounsaturated fatty acid levels than the other flood treatments; the other flood treatments were not different from each other or from the control treatment for this indicator. However, no differences in monounsaturated fatty acids were observed in the FTL samples. While Bossio and Scow (1998) found no differences in this indicator due to flood treatment or residue application, in subsequent studies a decrease in the monounsaturated fatty acids was seen with flooding conditions (Bossio et al., 2006). Other studies have shown that Gram-positive bacteria and Gram-negative bacteria respond to stress differently (Kieft et al., 1997). Both soils in this study (greenhouse and FTL field) had higher levels of Gram-positive bacteria than Gram-negative bacteria.

The microbial communities of the two different soil samples varied in their relationship with soil chemistry and C and N parameters. Microbial markers from the greenhouse experiment were generally not correlated with TOC, TN or C:N ratio. However,

microbial biomass, and the marker responses of aerobic bacteria, Gram-negative bacteria and Gram-positive bacteria from the FTL field study were all positively correlated with TOC and TN. In many cases the chemical characterization of the soils are given; however, study results are expressed in terms of treatment effects and direct relationships between PLFA results and soil chemistry measures are not given (i.e. direct correlations between soil N and microbial community structure are not made). Other studies examine changes in soil N and C in terms of fertilizer or management treatments (Acosta-Martinez et al., 1999; Bardgett et al., 1999; Clegg et al., 2003; Marschner et al., 2003). Bacterial biomass was weakly correlated with TN and bacteria and eukaryotic community structures were correlated with C:N ratio (Marschner et al., 2003). Bardgett et al. (2001) observed that total PLFA measures were negatively correlated to C:N ratio and B:F ratios were negatively correlated to N, but that PLFA evenness measures were positively related to TC and C:N. On the other hand, Clegg et al. (2003) found no relationship between PLFA community structure and TC or TN. Bardgett et al. (1999) concluded that PLFA patterns due to mineral-N availability were inconsistent. Further studies to clarify the relationships between soil characterization and soil microbial community analyses are undoubtedly needed.

5. Conclusions

This study illustrates the heterogeneous nature of the soil ecosystem. Treatment responses varied with environmental conditions and sampling depth. Responses to flood treatments were observed for microbial communities under simulated flood conditions in the greenhouse, and less so in the field. Stagnant flood conditions in the greenhouse decreased microbial biomass and markers of aerobic bacteria, Gram-negative bacteria, Gram-positive bacteria, and mycorrhizal fungi. Flooding in general, resulted in an increased response of the stress indicators, and the stagnant flood treatment resulted in a decreased response of monounsaturated fatty acids. Fluctuations in environmental conditions brought on by the intermittent flood treatment were expected to alter the soil microbial composition; however, this did not occur in all cases. The intermittent flood treatment increased the aerobic bacteria markers, but did not alter any other measurements. Total N, TOC and C:N ratio did not change with the flooding treatment compared to the control.

In the FTL field studies, flooding treatments reduced the ratios of bacteria to fungi and carbon to nitrogen. Biomass, aerobic bacterial, and Gram-positive bacterial and mycorrhizal markers increased with flooding. Stress indicators, monounsaturated fatty acids, TN and TOC were not affected by flooding treatment. Many of the differences in treatments were seen in the 10–20 cm depth. Stagnant conditions decreased B:F ratio, microbial biomass, aerobic bacterial, Gram-negative bacterial and Gram-positive bacterial markers, while stress indicators increased for the flowing flood treatment at the lower depth. No changes were seen in the monounsaturated fatty acid content in these studies. In the FTL field experiment, the flood treatments were less dramatic than that found in the greenhouse studies and were greater at the 10–20 cm depth than surface depths. As with previous studies, general decreases in microbial biomass and microbial marker response with depth were observed. Further studies with longer term flood treatments are needed to determine the effect of flooding disturbance on the character and function of soil microbial communities.

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References

Acosta-Martinez, V., Reicher, Z., Bischoff, M., Turco, R.F., 1999. The role of tree leaf mulch and nitrogen fertilizer on turfgrass soil quality. Biol. Fertil. Soils 29, 55–61.

Bailey, V.L., Peacock, A.D., Smith, J.L., Bolton Jr., H., 2002. Relationships between soil microbial biomass determined by chloroform fumigation–extraction, substrate-induced respiration, and phospholipid fatty acid analysis. Soil Biol. Biochem. 34, 1385–1389.

Baker III, T.T., Lockaby, B.G., Conner, W.H., Meier, C.E., Stanturf, J.A., Burke, M.K., 2001. Leaf litter decomposition and nutrient dynamics in four southern forested floodplain communities. Soil Sci. Soc. Am. J. 65, 1334–1347.

Baldwin, D.S., Mitchell, A.M., 2000. The effects of drying and re-flooding on the sediment and soil nutrient dynamics of lowland river-floodplain systems: a synthesis. Regulated Rivers: Res. Manage. 16, 457–467.

Balser, T.C., Treseder, K.K., Ekenler, M., 2005. Using lipid analysis and hyphal length to quantify AM and saprotrophic fungal abundance along a soil chronosequence. Soil Biol. Biochem. 37, 601–604.

Bardgett, R.D., Lovell, R.D., Hobbs, R.D., Jarvis, S.C., 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. Soil Biol. Biochem. 31, 1021–1030.

Bardgett, R.D., Jones, A.C., Jones, D.L., Kemmitt, S.J., Cook, R., Hobbs, P.J., 2001. Soil microbial community patterns related to the history and intensity of grazing in sub-montane ecosystems. Soil Biol. Biochem. 33, 1653–1664.

Belen Hinojosa, M., Carreira, J.A., Garcia-Ruiz, R., Dick, R.P., 2005. Microbial response to heavy metal-polluted soils: community analysis from phospholipid-linked fatty acids and ester-linked fatty acids extracts. J. Environ. Qual. 34, 1789–1800.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian J. Biochem. Physiol. 37, 911–917.

Bossio, D.A., Fleck, J.A., Scow, K.M., Fujii, R., 2006. Alteration of soil microbial communities and water quality in restored wetlands. Soil Biol. Biochem. 38, 1223–1233.

Bossio, D.A., Scow, K.M., 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microb. Ecol. 35, 265–278.

Bridge, J.S., 2003. Rivers and Floodplains: Forms, Processes and Sedimentary Record. Blackwell Science Ltd., Malden, MA, 260–295.

Cirtain, M.C., Franklin, S.B., Pezeshki, S.R., 2004. Effects of nitrogen and moisture regimes on *Arundinaria gigantean* (Walt.) Muhl. seedling growth. Nat. Areas J. 24, 251–257.

Clegg, C.D., Lovell, R.D.L., Hobbs, P.J., 2003. The impact of grassland management regime on the community structure of selected bacterial groups in soils. FEMS Microbiol. Ecol. 43, 263–270.

Drenovsky, R.E., Vo, D., Graham, K.J., Scow, K.M., 2004. Soil water content and organic carbon availability are major determinants of soil microbial community composition. Microb. Ecol. 48, 424–430.

Elhottova, D., Kristufek, V., Triska, J., Chrastny, V., Uhlirova, E., Kalcik, J., Picek, T., 2002. Immediate impact of the flood (Bohemia, August 2002) on selected soil characteristics. Water Air Soil Pollut. 173, 177–193.

Federle, T.W., 1986. Microbial distributions in soil—new techniques. Perspectives in Microbial Ecology. In: Meguser, M. (Ed.), Proceedings Fourth International Symposium in Microbial Ecology, American Society of Microbiology, Washington, D.C., pp. 493–498.

Fierer, N., Schimel, J.P., Holden, P.A., 2003. Variations in microbial community composition through two soil depth profiles. Soil Biol. Biochem. 35, 167–176.

Fraterrigo, J.M., Balser, T.C., Turner, M.G., 2006. Microbial community variation and its relationship with nitrogen mineralization in historically altered forests. Ecology 87, 570–579.

Fritze, H., Pietikainen, J., Pennanen, T., 2000. Distribution of microbial biomass and phospholipid fatty acids in Podzol profiles under coniferous forest. Eur. J. Soil Sci. 51, 565–573.

Frostegard, A., Baath, E., Tunlid, A., 1993. Shift in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. Soil Biol. Biochem. 25, 723–730.

Hackl, E., Pfeffer, M., Donat, C., Bachmann, G., Zechmeister-Boltenstern, S., 2005. Composition of the microbial communities in the mineral soil under different types of natural forest. Soil Biol. Biochem. 37, 661–671.

Ibekwe, A.M., Kennedy, A.C., 1998. Phospholipid fatty acid profiles and carbon utilization patterns for analysis of microbial community structure under field and greenhouse conditions. FEMS Microbiol. Ecol. 26, 151–163.

Joliffe, I.T., 1986. Principal Component Analysis. Springer-Verlag, New York.

Kieft, T.L., Wilch, E., O'Connor, K., Ringelberg, D.B., White, D.C., 1997. Survival and phospholipid fatty acid profiles of surface and subsurface bacteria in natural sediment microcosms. Appl. Environ. Microbiol. 63, 1531–1542.

Marschner, P., Kandeler, E., Marschner, B., 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. Soil Biol. Biochem. 35, 453–461.

Mentzer, J.L., Goodman, R.M., Balser, T.C., 2006. Microbial response over time to hydrologic and fertilization treatments in a simulated wet prairie. Plant Soil 284, 85–100.

Neatrour, M.A., Webster, J.R., Benfield, E.F., 2004. The role of floods in particulate organic matter dynamics of a southern Appalachian river-floodplain ecosystem. J. North Am. Benthological Soc. 23 (2), 198–213.

Olk, D.C., Cassman, K.G., Randall, E.W., Kinchesh, P., Sanger, L.J., Anderson, J.M., 1996. Changes in chemical properties of organic matter with intensified rice cropping in tropical lowland soil. Eur. J. Soil. Sci. 47, 293–303.

O'Leary, W.M., Wilkinson, S.G., 1988. Gram-positive bacteria. In: Rateledge, C., Wilkinson, S.G. (Eds.), Microbial Lipids. Academic Press, London, pp. 117–185.

Peacock, A.D., Mullen, M.D., Ringelberg, D.B., Tyler, D.D., Hedrick, D.B., Gale, P.M., White, D.C., 2001. Soil microbial community responses to dairy manure or ammonium nitrate applications. Soil Biol. Biochem. 33, 1011–1019.

Petersen, S.O., Frohne, P.S., Kennedy, A.C., 2002. Dynamics of a soil microbial community under spring wheat. Soil Sci. Soc. Am. J. 66, 826–833.

Petersen, S.O., Klug, M.J., 1994. Effects of sieving, storage, and incubation temperature on the phospholipid fatty acid profile of a soil microbial community. Appl. Environ. Microbiol. 60, 2421–2430.

Ratledge, C., Wilkinson, S.G., 1988. Microbial Lipids, vol. 1. Academic Press, London.

SAS, 2002. SAS STAT User's Guide. SAS Institute Inc., Cary, NC.

Schuur, E.A.G., Matson, P.A., 2001. Net primary productivity and nutrient cycling across a mesic to wet precipitation gradient in Hawaiian montane forest. Oecologia 128, 431–442.

Sundh, I., Nilsson, M., Borga, P., 1997. Variation in microbil community structure in two boreal peatlands as determined by analysis of phospholipid fatty acid profiles. Appl. Environ. Microb. 63, 1476–1482.

Suzuki, C., Kunito, T., Aono, T., Liu, C.-T., Oyaizu, H., 2005. Microbial indices of soil fertility. J. Appl. Microbiol. 98, 1062–1074.

Tabachnick, B.G., Fidell, L.S., 2001. Using Multivariate Statistics, 4th edition. Pearson Education Company, Needham Heights, MA.

Tsutsuki, K., Ponnamperuma, F.N., 1987. Behavior of anaerobic decomposition products in submerged soils. Soil. Plant Nutr. 33 (1), 13–33.

Vepraskas, M.J., Faulkner, S.P., 2001. Redox chemistry of hydric soils. In: Richardson, J.L., Vepraskas, M.J. (Eds.), Wetland Soils: Genesis, Hydrology, Landscapes and Classification. Lewis Publishers, Boca Raton, Florida.

Vestal, J.R., White, D.C., 1989. Lipid analysis in microbial ecology. Bioscience 39, 535–541.

Wander, M.M., Hedrick, D.S., Kaufman, D., Traina, S.J., Stinner, B.R., Kehrmeyer, S.R., White, D.C., 1995. The functional significance of the microbial biomass in organic and conventionally managed soils. In: Collins, H.P., Robertson, G.P., Klug, M.J. (Eds.), The Significance and Regulation of Soil Biodiversity. Kluwer Academic Publishers, Netherlands, pp. 87–97.

Zelles, L., Bai, Q.Y., Rackwitz, R., Chadwick, D., Beese, F., 1995. Determination of phospholipid- and lipopolysaccharide-derived fatty acids as an estimate of microbial biomass and community structures in soils. Biol. Fertil. Soils. 19, 115–123.